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review

The Genetics and Molecular Biology of Flocculation in *Saccharomyces cerevisiae*: An Overview

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Summary

Floculation in Sacch. cerevisiae is a process of reversible cellular aggregation. Their ability to flocculate enables efficient separation of cells from the fermentation medium and facilitates subsequent downstream processing of the product. The most important advances in our knowledge have been achieved about the mechanisms of flocculation and its genetic bases. Recent advances in both fields are reviewed and some possible hints for the future are given.

Keywords: flocculation; *Saccharomyces cerevisiae;* phenotype; genes; control of

Introduction

Flocculation in *Saccharomyces cerevisiae* is a process of reversible cellular aggregation by which yeast cells adhere to form clumps that spontaneously sediment in the medium in which they are suspended. This phenomenon depends on calcium and is sensitive to the action of mannose and protease. This feature has been shown to be a completely different type of aggregation to that originated by the mating of haploid strains and of the cellular groupings that form during bud separation from mother cells *(1)*. The phenomenon of flocculation is a highly complex process influenced by genetic and environmental factors, thus rendering its study particularly difficult (for a recent review see reference 2).

Although this character has been studied in some depth in *Sacch. cerevisiae*, it has also been described in other yeast species such as *Hansenula anomala (3); Kluyveromyces bulgaricus (4); Kluyveromyces marxianus (5); Pichia pastoris (6); Schizosaccharomyces pombe (7)* and *Candida famata (8)*.

Because their ability to flocculate enables efficient separation of cells from the fermentation medium, this characteristic is a suitable property in yeast strains involved in certain industrial fermentation processes such as brewing, wine making, and champagne and cava production. It would also be of interest in the production of yeast biomass (single cell protein) and in modern biotechnology owing to the ever increasing use of the ascomycetous yeast *Sacch. cerevisiae* in the production of heterologous proteins. Likewise, it is considered to be an important characteristic in the development of continous fermentation processes (such as the biological production of ethanol) since highly flocculent strains make it possible to use high cell concentrations in the fermentor, this being favourable for high yields *(9)*.

In sum, in all these processes it is necessary to separate the cells from the final product or from the culture medium, flocculation has become a low cost method to achieve this goal because it facilitates subsequent downstream processing of the product. Owing to the commercial interest in flocculation, this characteristic has received considerable attention in recent years. The most important advances have been achieved in our knowledge of the mechanism of flocculation and its genetic bases. This paper reviews recent progress in both fields and offers possible hints for the future.

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The Flocculation Mechanism

Our knowledge about the flocculation mechanism, as currently accepted, comes straight from studies on the effect of calcium as well as proteases and sugars on the phenomenon.

Effect of inorganic salts. The requirement in inorganic ions for flocculation to take place in yeasts has been described by many authors who have demonstrated the effect of chlorides and different sulphates (calcium, sodium, magnesium and potassium) on flocculation *(10,11)*. Whereas some strains may be deflocculated by eliminating these ions by simply rinsing the cells in distilled water *(12)*, others require the presence of chelating agents such as EDTA *(13)*; in all cases the addition of small amounts of calcium, magnesium or manganese suffices to reestablish flocculation *(14)*. Although there are a number of ions which may promote flocculation, it is generally accepted that calcium is by far the most effective. It has also been shown that calcium analogues, such as strontium or barium, competitively inhibit flocculation *(13,15)*, and it has been found that there are other salts which indirectly promote flocculation by inducing the leakage of intracellular calcium *(16)*. Besides the effect of salts on stimulating and inhibiting flocculation, they also probably play a role in developing flocculation; in this sense it has been reported that phosphate is necessary to promote flocculation *(17)*, whereas it may be inhibited in magnesium-lacking environments *(18)*.

Based on the effect of calcium, a theory was proposed to explain the phenomenon of flocculation known as the calcium-bridging hypothesis *(19,20)*. According to this theory, flocculation is the result of links formed by bivalent calcium cations between yeast cells, supported by hydrogen bridges. The surface groups interacting with the calcium would be carboxyl groups *(20)* or phosphodiester groups in the cell wall mannan *(21,22)*.

Effect of proteases and protein-denaturing agents. Eddy and Rudin *(23)* showed that the treatment of flocs with papain elicits an irreversible loss of flocculation, thus implicating cell surface proteins in the flocculation process. Subsequently, it was observed that other proteases such as pronase E, proteinase K, trypsin, chymotrypsin and pepsin, besides certain protein denaturing agents such as mercaptoethanol, urea and guanidine, also inhibit this character in an irreversible manner *(24,25)*. Treatment with proteases also allowed differentiation between different types of flocculation *(26)*. These results, which clearly implicated surface cell proteins in the flocculation process, were consistent with the calcium-bridging theory.

Effect of sugars on flocculation. Flocculation in yeasts may be inhibited by different sugars such as mannose, maltose, saccharose and glucose *(10,20,27–29)*. This cannot be explained by the calcium-bridging hypothesis alone. Thus, Taylor and Orton *(30)*, and in particular Miki *et al. (31,32)* proposed an additional (now widely accepted) theory to explain flocculation, known as the lectin-like theory. This theory is based on the fact that flocculation is sensitive to the effect of proteases and that the possible protein involved shows the basic properties of lectins, i.e. proteins which link to specific sugars *(33)* and that require metal ions to do so. In keeping with this hypothesis, flocculation would occur by surface cell proteins of the flocculent cells (similar to lectins) joining the α-mannan carbohydrates of adjacent cells (Fig. 1A). This idea is consistent with previous results from studies on co-flocculation between flocculent and non-flocculent strains sharing the existence of two different parts in the flocculent links: a protein and a receptor *(34,35)*. The notion is also consistent with the fact that the lectins of flocculent strains are sensitive to proteases, whereas the receptors present in both flocculent and non-flocculent cells are insensitive to such treatment. The role of calcium, in this theory, would be to maintain lectins in the correct conformation *(36)*.

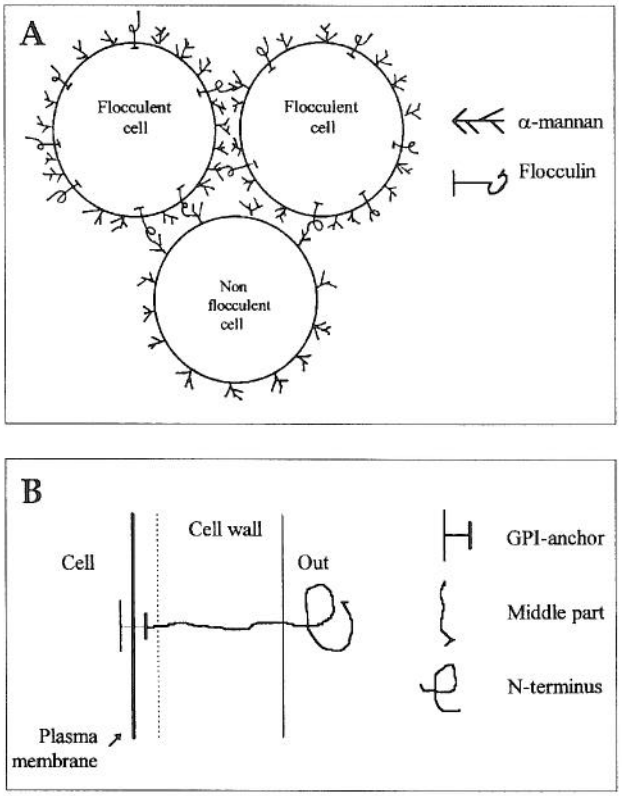

Fig. 1. Lectin-like theory of flocculation. A) Flocculation between flocculent and non-flocculent (coflocculation) cells. Proteins on the cell surface bind to the α-mannan carboydrates in adjacent cells (based on the model of Miki *et al.*,1982; reference *31*). B) Flocculin structure according to the *FLO1* sequence (based on the model of Teunissen *et al.*, 1993; reference *81*).

Although some authors have observed a specific inhibition of flocculation with mannose *(30,31)*, others have reported inhibition with several sugars *(20,27)*. Thus in comparative studies with a large number of brewer's yeasts and laboratory strains, Stratford noted two different groups of flocculent strains *(37)*. One group could only be dispersed by mannose, whereas in the other group flocculation could be inhibited by mannose, maltose, saccharose and glucose. Both types of strains could also be differentiated by phenotype inhibition in the presence of particular concentrations of salt and certain pH values, their selective sensitivity to the

effect of different proteases, the growth phase and the environment in which flocculation is expressed. The first group (called the Flo1 phenotype) encompasses strains whose flocculation is due to the *FLO1*, *FLO5*, *tup1* and *ssn6* genes, whereas the second type (called the NewFlo phenotype) groups all brewer's yeast strains *(28,37)*. According to the authors, these two different phenotypes represent the expression of two different surface lectins. However, studies performed on wine flocculent strains, whose flocculent phenotype has been shown to be due to the *FLO1* gene *(29)*, were unable to definitively classify them under either of the above two phenotypes, thus throwing doubt on the aforementioned proposal of the existence of two different lectins to account for the differential flocculation observed. It is more likely that the flocculation mechanism would be essentially the same for all flocculent strains and that the observed differences would be due to other factors, such as timing of cell surface hydrophobicity changes as clearly shown by Straver and coworkers *(38,39)*. It should be noted that besides the two groups of strains reported above, a third type has been described which is neither sensitive to mannose nor to calcium *(40)*. In this latter case, the flocculation mechanism would probably be completely different.

Genetic and Molecular Bases of Flocculation

FLO genes. Flocculation in *Sacch. cerevisiae* is genetically controlled (Table 1). The genetic basis of the character has been independently reported by several laboratories, including our own. The results are unambiguous in that there must be a very complex genetic mechanism underlying yeast flocculation. According to Gilliland *(41)*, the flocculent character of a strain would be due to a single gene, whereas according to Thorne *(42,43)*, flocculation would involve 3 loci not linked in the same strain. Subsequent studies (using brewer's strains) described 3 genes with the ability to originate a flocculent phenotype; two of these were dominant (*FLO1* and *FLO2*) and the third recessive, known as *flo3 (44,45)*. Almost at the same time, a further dominant flocculation gene was discovered and named *FLO4*. This gene was mapped on chromosome I of *Sacch. cerevisiae* at 37 cM from the *ade1* marker *(46,47)*. It was later shown that the *FLO1*, *FLO2* and *FLO4* genes were allelic *(48)* and the locus was called *FLO1*. The position of this gene was confirmed by several authors: thus Skatrud *et al. (49)* mapped it at 41.8 cM from *ade1*, Teunissen and Steensma *(50)* located it at 24 Kb from the right end of chromosome I and Sieiro *et al. (51)* at 4.7 cM from *PHO11*.

Another flocculation gene, non allelic with the previous ones, was characterized by Johnston and Reader *(52)* and was named *FLO5*. The flocculation of these strains was differentiated from flocculation in strains containing the *FLO1* gene by Hodgson *et al. (26)*. According to these authors, the flocculent phenotype of *FLO1* strains is resistant to temperature (70 °C) and sensitive to treatment with chymotrypsin, whereas strains characterized as *FLO5* behave in the opposite manner. The *FLO5* gene remained unmapped by classical genetic techniques for many years. First, it was erroneously mapped on chromosome I *(53)*. Later, more in-depth analysis of the *FLO5* strains showed that its flocculent phenotype was the result of two different genes: one of these was allelic with the previously described *FLO1*, and the other (*FLO5*) was mapped on chromosome VIII at 36.8 cM from the *PET3* gene and 30.5 cM from *FUR1*. The latter gene originated a dominant and constitutive flocculent character *(54,55)*.

The *FLO8* gene was first described by Yamashita and Fukui *(56)* and was mapped on chromosome VIII, linked to the marker *arg4*. These results contrast with those reported by Teunissen *et al. (54)* who, assuming that *FLO8* was similar to *FLO1*, mapped the *FLO8* gene on chromosome I by genic disruption (using a plasmid based on the *FLO1* gene), and concluded that it was allelic with *FLO1*. Later, cloning and sequencing of *FLO8* showed that it was not an allele of *FLO1* and that it differed significantly in its sequence. Northern analysis showed that *FLO8* mediates flocculation by activating the transcription of *FLO1*. *FLO8* is also required for diploid pseudohyphal growth and haploid invasive growth. Regarding its localization, the gene sequence showed high homology with chromosome V of *Sacch. cerevisiae (57,58)*.

Sequence analysis of the genome of *Sacch. cerevisiae* together with Southern hybridization studies on karyo-

Table 1. *FLO* genes and their suppressors

Gene name	Localization	Characteristics	Refs.
FLO1	Chromosome I	Structural, dominant	*48–51*
FLO2	Chromosome XII	Regulator	*63*
flo3		Semi-dominant	*44,45*
FLO5	Chromosome VIII	Structural, dominant	*52,54,55*
flo6		Recessive	*52*
flo7		Recessive	*52*
FLO8	Chromosome V	Flocculation activator	*56–58*
FLO9	Chromosome I	Structural	*59*
FLO10	Chromosome XI	Structural	*59*
FLO11	Chromosome IX	Structural, dominant	*61*
fsu1		Flocculation suppressor	*66*
fsu2		Flocculation suppressor	*66*
fsu3		Flocculation suppressor	*50*
sfl1		Flocculation suppressor	*70,71*

Table 2. Summary of other genes involved in flocculation

Gene name	Allele	Characteristics	Refs.
tup1	*aar1, aer2, amm1, cyc9, flk1, sfl2, umr7*	Transcription repression	*67,69*
cyc8	*ssn6*	Transcription repression	*68,69*
pho2		Transcription activator	*60*
MIG1		Flocculation repression	*85,86*
LSR1		Transcription factor	*59*
FH4C		Constitutive invertase producer	*73*
cka2		Casein Kinase II	*72*
wal		Wall morphology	*77*
abs		Acid phosphatase	*77*
kre6		(1–6)β-glucan synthase	*78*
skn1		(1–6)β-glucan synthase	*78*
HTLV1-Tax		Transactivator	*74*
Ha-ras		Human *Ha-ras*	*75*

types using the *FLO1* gene as probe confirmed the presence and position of the genes described above *(59)*. Thus, according to the complete sequencing of chromosome I, *FLO1* is found at the expected position. In internal regions the gene comprises 5 repeated units named A, B, C, D and E. The different clones of the *FLO1* gene showed differences in their sequences that consisted in deletions localized in this repeated region. Approximately 10 Kb downstream from *FLO1*, a homologous sequence appears; this has been considered a pseudogene because it has stop codons in different positions. Another possible flocculation gene is present on chromosome I near the end of the left arm. This shows 94% similarity at protein level with the *FLO1* gene. It appears to be a true flocculation gene and has been named *FLO9*. A second pseudogene has also been located on the left arm of chromosome I.

Likewise, the above analysis confirmed the presence of the *FLO5* gene on chromosome VIII. The product of this gene shows 96% similarity with the product codified by *FLO1*. Again, in this case, a pseudogene was detected 10 Kb downstream from *FLO5*.

The genes *FLO1*, *FLO5* and *FLO9* differ in the length of the repeated unit A in their central part (18 times for *FLO1*, 8 times for *FLO5*, and 14 times for *FLO8*). According to Northern analysis carried out by Teunissen and Steensma *(59)*, the *tup1* and *ssn6* mutants exhibiting a flocculent phenotype (see below) show three transcripts which hybridize with the *FLO1* probe, whose sizes are 4.8, 4.2 and 3.5 Kb. According to these authors, the 4.8 Kb transcript corresponds to the *FLO1* gene; the 3.5 one to *FLO5*, and the 4.2 transcript must correspond to the *FLO9*, taking into account the differences that the three genes display in their central part (unit A).

The sequence of chromosome XI also shows homology with the *FLO* genes at the N- and C-terminus. The putative protein codified for this gene (*FLO10*) shows 58% similarity with the *FLO1* product *(59)*. The expected transcript for this gene is 3.7 Kb. A transcript with this length appears together with that of 4.8 Kb in *pho2* mutants *(60)*, which also show a flocculent phenotype (see below).

In addition to the above genes, Wan-Sheng and Dranginis *(61)* characterized another flocculation gene (*FLO11*) that is related to the *STA* genes which induce calcium-dependent cellular aggregation when expressed in yeasts. *FLO11* is located on chromosome IX and encodes for a flocculine with 37% similarity with the product of *FLO1*. Unlike the other flocculation genes, the *FLO11* gene is located near the centromere rather than close to the telomere.

A gene located on chromosome XII that is able to originate a remarkable flocculent phenotype when overexpressed in non-flocculent strains and in a mutant affected in the *FLO1* locus has also been described *(62,63)*. The gene has been called *FLO2*, although no homology with the other *FLO* genes has been observed. *FLO2* must activate the expression of one of the erlier described flocculation genes.

Apart from the dominant flocculation genes referred to so far, several semidominant or recessive flocculation genes have been characterized: *flo3* *(44,45)*, *flo6* and *flo7* *(52)*. Since it is currently known that the non-flocculent strains have flocculation genes that are not expressed in certain genetic backgrounds, it may be assumed that *flo3*, *flo6* and *flo7* are allelic with some of the flocculation genes described above.

Flocculation suppressor genes. The instability of the flocculent character has been noted both in industrial beer strains and in genetic laboratory *FLO1* strains *(48,64)*. In diploid strains, the suppression of flocculation may be due to the regulation of the phenotype by the mating type *(65)*. Also, suppression of flocculation is coded by specific genes (Table 1). Initially, two genes called *fsu1* and *fsu2* which suppress the flocculent phenotype in *FLO1* strains were found *(66)*. In a subsequent study, a third flocculation suppressor, proposed as *fsu3*, was described; this is widely distributed among the non-flocculent strains. This gene suppresses the flocculent character in the strain IM1-8b (used in that study) but has no effect on other strains characterized as *FLO1* or *FLO5* *(50,54)*. The manner in which these genes suppress flocculation remains to be clarified.

Other genes responsible for flocculation. Besides the *FLO* and their suppressor genes, a series of mutations in other regulatory genes also originate a flocculent phenotype in *Sacch. cerevisiae* (Table 2). Among these, the best studied are the mutations in the *TUP1* and *SSN6* genes (or their alleles) which cause flocculation similar to that conferred by the *FLO* genes, apart from other pleiotropic effects *(67,68)*. *TUP1* and *SSN6* are regulatory genes involved in inhibiting transcription activators *(69)*. Similarly, the loss of activity of other genes that are non-allelic with the above ones, such as *SFL1*, Table 1 *(70, 71)*, *CKA2* *(72)*, *FH4C* *(73)* and *PHO2* *(60)*, also gives rise to flocculation.

Overexpression of the *LSR1* transcription factor of *Sacch. cerevisiae* *(59)* also confers a flocculent phenotype similar to that of *FLO1* strains. The same occurs with the heterologous transcription activator HTLV1–Tax when expressed in *Sacch. cerevisiae* *(74)* and also with the human gene Ha-ras *(75)*.

Likewise, mutations in different genes involved in cell wall synthesis (*wal*, *abs*, *kre6* and *skn1*) also originate flocculation. Furthermore, these mutations increase the excretion of invertase, acid phosphatase and melibiase, and alter cell morphology *(76–78)*. The way in which these genes induce cellular aggregation remains unclear since they are not regulatory genes and must necessarily be related to the overall cell architecture. Additionally, some authors have suggested that alterations in these genes may render cells more susceptible to responses to stress or, alternatively, modifications in the cell wall that may help the flocculines to reach the cell surface *(59)*.

Structural or regulatory nature of flocculation genes. Non-flocculent strains with a mutation in their regulatory genes such as *TUP1* or *SSN6* show a flocculent phenotype similar to those having an active *FLO* gene. In view of this, it has been suggested *(i)* that the structural gene encoding flocculation lectins is probably common and is present in all strains of *Sacch. cerevisiae*, and *(ii)* that *flocculation* genes, like *TUP1* and *SSN6*, would be regulatory genes that activate the expression of a hitherto unidentified structural flocculation gene *(1)*.

However, molecular studies on *FLO* genes (*FLO1*, *FLO5*, *FLO9* and *FLO11*) now make it possible to consider them as structural genes. The *FLO1* gene has been cloned *(79,80)* and sequenced *(81,82)* by several research groups; the gene is of 4.5 Kb and codes for a 150 KDa protein. As regards the *FLO1* sequence, it has been concluded that the protein codified by the gene starts with a hydrophobic secretory signal sequence and belongs to the class of GPI-anchored serine/threonine-rich cell wall proteins. The protein would be located with the N-terminus exposed to the medium *(81,82)* (Fig. 1B). These data have been confirmed by other authors. Thus, Bidard *et al. (83)* using FITC-labelled antibodies against the N-terminus part of the protein, demonstrated its presence in the cell wall. The same results were obtained when the N-terminus of the protein codified by *FLO11* was fused with the GFP (Green Fluorescent Protein) which is able to produce fluorescence *in vivo* without cofactors; fluorescence appears externally surrounding the cell *(61)*.

The fact that the protein is localized in the cell wall and that no DNA binding motifs – *i.e.* helix-loop-helix, leucine zipper or Zn-finger – are present in the sequence strongly suggests a direct role of *FLO* genes in flocculation. *FLO* genes must therefore be structural flocculation genes. In some strains they are expressed constitutively while in others they are regulated (at least *FLO1*, *FLO5* and *FLO9*) by *TUP1* and *SSN6*, which form part of a regulatory cascade. *TUP1* and *SSN6* are involved in the repression of many important genes in yeasts. It has been suggested that Tup1p-Ssn6p locates the genes that it represses by recognizing the specific DNA-bound proteins present in each promoter *(84)*. Recently, Shankar *et al. (85)* have proposed that a good candidate for such a protein would be the product of the *MIG1* gene: a C_2H_2 Zinc-finger DNA-binding protein with domains for Tup1p and Ssn6p *(86)*. It was found that *MIG1* plays a role in flocculation. Disruption of *MIG1* in a flocculent strain or in a *tup1* mutant results in a non-flocculent phenotype and overexpression of the gene causes strong flocculation *(85)*. According to those authors *MIG1* has a probable repressor function in flocculation gene expression. An activator (*FLO8* has been decribed as an activator of flocculation genes) could be controlled by an active repressor whose function is repressed by *MIG1*.

Flocculation is thus a crucial property in almost all yeasts used in many industrial processes, specially when it is expressed in the late exponential phase of growth. However, more work is necessary to confirm the proposed model and to finally clarify how flocculation is regulated; this would be of great interest to control the character in industrial strains.

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References

1. M. Stratford, *Yeast, 8* (1992) 25–38.
2. M. Stratford, *Coll. Surf. B: Biointerfaces, 2* (1994) 151–158.
3. K. Saito, S. Sato, H. Shimoi, H. Iefuji, H. Tadenuma, *Agric. Biol. Chem. 54* (1990) 1425–1432.
4. T. Hussain, O. Salhi, J. Lematre, C. Charpentier, R. Bonaly, *Appl. Microbiol. Biotechnol. 23* (1986) 269–273.
5. P. Bajpai, A. Margaritis, *Biotechnol. Bioeng. 28* (1986) 283–287.
6. A. Mbawala, S. AlMahmood, V. Loppinet, R. Bonaly, *J. Gen. Microbiol. 136* (1990) 1279–1284.
7. B. F. Johnson, T. Walker, G. B. Calleja, V. Seligy, *Can. J. Microbiol. 34* (1988) 1105–1107.
8. X. Cubells Martínez, A. Narbad, A. T. Carter, M. Stratford, *Yeast, 12* (1996) 415–423.
9. K. Esser, U. Kues, *Process Biochem. 18* (1983) 21–23.
10. J. A. Burns, *J. Inst. Brew. 43* (1937) 31–43.
11. H. E. Jansen, Flocculation in yeasts. In: *The Chemistry and Biology of Yeasts*, A. H. Cook (Ed.), Academic Press Inc., New York (1958) pp. 635–667.
12. H. E. Jansen, F. Mendlik, *Proceedings of the European Brewery Convention Congress*, Brighton (1951) p. 59.
13. N. W. Taylor, W. L. Orton, *J. Inst. Brew. 79* (1973) 294–297.
14. N. W. Taylor, W. L. Orton, *J. Inst. Brew. 81* (1975) 53–57.
15. H. Nishihara, T. Toraya, S. Fukui, *Arch. Microbiol. 131* (1982) 112–115.
16. M. Stratford, *Yeast, 5* (1989) 487–496.
17. A. A. Eddy, A. D. Rudin, *J. Inst. Brew. 64* (1958) 139–142.
18. H. Nishihara, T. Toraya, S. Fukui, *J. Ferment. Technol. 54* (1976) 356–360.
19. J. O. Harris, *J. Inst. Brew. 65* (1959) 5–6.
20. P. J. Mill, *J. Gen. Microbiol. 35* (1964) 61–68.
21. T. P. Lyons, J. S. Hough, *Brew. Dig. 45* (1970) 52–60.
22. T. P. Lyons, J. S. Hough, *J. Inst. Brew. 77* (1971) 300–305.
23. A. A. Eddy, A. D. Rudin, *J. Inst. Brew. 64* (1958) 19–21.
24. G. G. Stewart, I. Russell, I. F. Garrison, *Proceedings of the American Society of Brewing Chemists* (1973) pp. 100–106.
25. H. Nishihara, T. Toraya, S. Fukui, *Arch. Microbiol. 131* (1982) 112–115.
26. J. A. Hodgson, D. R. Berry, J. R. Johnston, *J. Gen. Microbiol. 131* (1985) 3219–3227.
27. A. A. Eddy, *J. Inst. Brew. 61* (1955) 313–317.
28. M. Stratford, S. Assinder, *Yeast, 7* (1991) 559–574.
29. C. Sieiro, N. M. Reboredo, T. G. Villa, *J. Ind. Microbiol. 14* (1995) 461–466.
30. N. W. Taylor, W. L. Orton, *J. Inst Brew. 84* (1978) 113–114.
31. B. L. A. Miki, N. H. Poon, A. P. James, V. L. Seligy, *J. Bacteriol. 150* (1982) 878–889.
32. B. L. A. Miki, N. H. Poon, V. L. Seligy, *J. Bacteriol. 150* (1982) 890–899.
33. W. C. Boyd, E. Shapleigh, *Science, 119* (1954) 419.
34. G. G. Stewart, *MBAA Tech. Quart. 9* (1972) 25.
35. G. G. Stewart, Y. F. Garrison, *Proceedings of the American Society of Brewing Chemists* (1972) pp. 118–131.
36. N. Sharon, H. Lis, *Science, 117* (1972) 949–959.
37. M. Stratford, *Yeast, 5* (1989) 441–445.
38. M. H. Straver, J. W. Kijne, G. Smit, *Tibtech. 11* (1993) 228–232.
39. M. H. Straver, V. M. Traas, G. Smit, J. W. Kijne, *Yeast, 10* (1994) 1183–1193.
40. C. L. Masy, A. Henquinet, M. M. Mestdagh, *Can. J. Microbiol. 38* (1992) 1298–1306.

41. R. B. Gilliland, *Proceedings of the European Brewery Convention Congress*, Brighton (1951) pp. 35–58.
42. R. S. W. Thorne, *Proceedings of the European Brewery Convention Congress*, Brighton (1951) pp. 21–24.
43. R. S. W. Thorne, *Compt-rend. Lab. Calsberg. Serv. Physiol. 25* (1951) 101–140.
44. J. R. Johnston, C. W. Lewis, Genetic analysis of flocculation in *Saccharomyces cerevisiae* and tetrad analysis of commercial brewing and baking strains. In: *Second International Symposium on the Genetics of Industrial Microorganisms*, K. D. MacDonald (Ed.), Academic Press, London (1974) pp. 339–355.
45. C. W. Lewis, J. R. Johnston, P. A. Martin, *J. Inst. Brew. 82* (1976) 158–160.
46. G. G. Satewart, I. F. Garrison, T. E. Goring, M. Meleg, P. Pipasts, I. Russell, *Kemia-Kemi, 3* (1976) 465–479.
47. G. G. Stewart, I. Russell, *Can. J. Microbiol. 23* (1977) 441–447.
48. I. Russell, G. G. Stewart, H. P. Reader, J. R. Johnston, P. A. Martin, *J. Inst. Brew. 86* (1980) 10–12.
49. P. L. Skatrud, E. J. Kot, J. R. Helbert, *ASBC J. 40* (1982) 52–55.
50. A. W. R. H. Teunissen, H. Y. Steensma, *Yeast, 6* (1990) 35–39.
51. C. Sieiro, E. Longo, J. Cansado, J. B. Velázquez, P. Calo, P. Blanco, T. G. Villa, *FEMS Microbiol. Lett. 112* (1993) 25–30.
52. J. R. Johnston, H. P. Reader, Genetic control of flocculation. In: *Yeast Genetics. Fundamental and Applied Aspects*, J. F. T. Spencer, D. M. Spencer, A. R. W. Smith (Eds.), Springer-Verlag, New York, Berlin, Heidelberg, Tokyo (1983) pp. 205–224.
53. F. Vezinhet, B. Blondin, P. Barre, *Biotechnol. Lett. 13* (1991) 47–52.
54. A. W. R. H. Teunissen, J. A. Van Den Berg, H. Y. Steensma, *Yeast, 11* (1995) 735–745.
55. C. Sieiro, N. M. Reboredo, P. Blanco, T. G. Villa, *Microbiología SEM, 12* (1996) 449–456.
56. I. Yamashita, S. Fukui, *Agric. Biol. Chem. 47* (1983) 2889–2896.
57. O. Kobayashi, H. Suda, T. Ohtani, H. Sone, *Mol. Gen. Genet. 251* (1996) 707–715.
58. H. Liu, C. A. Styles, G. R. Fink, *Genetics, 144* (1996) 967–978.
59. A. W. R. H. Teunissen, H. Y. Steensma, *Yeast, 11* (1995) 1001–1013.
60. G. Berben, M. Legrain, F. Hilger, *Gene, 66* (1988) 307–312.
61. Wan-Sheng Lo, A. M. Dranginis, *J. Bacteriol. 178* (1996) 7144–7151.
62. N. M. Reboredo, C. Sieiro, P. Blanco, T. G. Villa, *FEMS Microbiol. Lett. 137* (1996) 57–61.
63. C. Sieiro, N. M. Reboredo, P. Blanco, T. G. Villa, *FEMS Microbiol. Lett. 146* (1997) 109–115.
64. G. G. Stewart, *Brew. Dig. 50* (1975) 42–56.
65. J. Watari, M. Kudo, N. Nishikawa, M. Kamimura, *Agric. Biol. Chem. 54* (1990) 1677–1681.
66. S. Holmberg, M. C. Kielland-Brandt, *Calsberg Res. Commun. 43* (1978) 37–47.
67. P. N. Lipke, C. Hull-Pillsbury, *J. Bacteriol. 159* (1984) 797–799.
68. R. J. Trumbly, *Gene, 73* (1988) 97–111.
69. F. E. Williams, R. J. Trumbly, *Mol. Cell Biol. 10* (1990) 6500–6511.
70. A. Fujita, Y. Kikuchi, S. Kuhara, S. Matsumoto, H. Kobayashi, *Gene, 85* (1989) 321–328.
71. K. Taira, M. Oda, H. Shinshi, H. Maeda, K. Furukawa, *Protein Eng. 3* (1990) 733–737.
72. R. Padmanabha, J. L.-P. Chen-Wu, D. E. Hanna, C. V. C. Glover, *Mol. Cell. Biol. 10* (1990) 4089–4099.
73. B. S. Montenecourt, S. C. Kuo, J. O. Lampen, *J. Bacteriol. 114* (1973) 233–238.
74. R. A. Kramer, L. Tomchak, S. M. Ruben, C. A. Rosen, *Aids Res. Hum. Retroviruses, 6* (1990) 1305–1309.
75. S. Clark, J. P. McGrath, A. D. Levinson, *Mol. Cell Biol. 5* (1985) 2746–2752.
76. P. E. Hansche, *Genetics, 79* (1975) 661–674.
77. P. Lange, *Mol. Gen. Genet. 173* (1979) 211–212.
78. T. Roemer, S. Delaney, H. Bussey, *Mol. Cell. Biol. 13* (1993) 4039–4048.
79. J. Watari, Y Takata, M. Ogawa, N. Nishikawa, M. Kamimura, *Agric. Biol. Chem. 53* (1989) 901–903.
80. A. W. R. H. Teunissen, J. A. van den Berg, H. Y. Steensma, *Yeast, 9* (1993) 1–10.
81. A. W. R. H. Teunissen, E. Holub, J. Van den Hucht, J. A. van den Berg, H. Y. Steensma, *Yeast, 9* (1993) 423–427.
82. J. Watari, Y. Takata, M. Ogawa, *et al.*, *Yeast, 10* (1994) 211–225.
83. F. Bidard, M. Bony, B. Blondin, S. Dequin, P. Barre, *Yeast, 11* (1995) 809–822.
84. C. A. Keleher, M. J. Redd, J. Schultz, M. Calrson, A. D. Johnson, *Cell, 68* (1992) 709–719.
85. C. S. Shankar, M. S. Ramakrishnan, S. Umesh-Kumar, *Microbiology, 142* (1996) 2663–2667.
86. M. A. Treitel, M. Carlson, *Proc. Natl. Acad. Sci. USA, 92* (1995) 3132–3136.

Pregled genske i molekularne biologije flokulacije u *Sacch. cerevisiae*

Sažetak

Flokulacija u Sacch. cerevisiae je reverzibilni proces agregacije stanica. Njihova sposobnost da flokuliraju omogućuje djelotvorno odvajanje stanica od fermentacijske podloge i olakšava izdvajanje proizvoda u daljnjem tijeku prerade. Najvažniji napredak postignut je u razumijevanju mehanizma flokulacije i njegove genetičke osnove. U članku su prikazana najnovija dostignuća u oba područja i dane neke sugestije za daljnja istraživanja.